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**Original Article** 

# Genetic variation of improved oil palm *tenera* hybrid populations using morphological and SSR markers

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### Abstract

Genetic variation of the *tenera* hybrid progenies provides useful guidance to the management of appropriate breeding programs, especially those for development of high-yielding *tenera* varieties. Such programs involve costly and time-consuming adaptability and stability tests. In this present study, twelve  $F_1$  *tenera* hybrid populations derived from *dura×pisifera* crosses were assessed for genetic variation based on seedling morphological traits and SSR markers. According to the morphological characters evaluated, P1, P4, and P13 showed more within population variation than the others. PCA analysis indicated that the progenies with large PC1 and PC2 components exhibited potential for high productivity, and the corresponding parental *dura* and *pisifera* should be selected for further breeding. SSR analysis revealed that P1, P4, P6 and P8 were the most genetically diverse *tenera* hybrid populations, indicating high potential for adaptation and yield stability. Consequently, P1, P4, P6, P8 and P13 populations should be included in further breeding efforts.

Keywords: oil palm, genetic variation, morphology, SSR markers, hybrid population

## 1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) is an economically important oil crop and has become one of the main oil crops worldwide. The major oil palm producing countries are Indonesia and Malaysia, which account for more than 80 percent of the global share (Otieno *et al.*, 2016). Thailand ranked in the third place with the planted area of 636,800 ha in 2013, contributing approximately 3 of the global production (Taeprayoon, Tanya, Lee, & Srinives, 2015). The majority of oil palm growing area in Thailand is in the southern peninsular region, particularly in Phang-nga, Krabi, Surat Thani and Chumphon provinces owing to their geographical advantages (Colchester *et al.*, 2011). However, due to promotion by the Thai national government, oil palm plantation area has rapidly expanded also to other provinces and regions and is expected to reach 1.6 million ha by 2029. Along with

\*Corresponding author Email address: saowapa.d@psu.ac.th the increasing plantation area, germplasm improvements must be considered. In the commercial production of oil palm, breeding of cultivars for high oil content, slow vertical stem growth, resistance to diseases, and better oil quality are pursued (Corley & Tinker, 2003; Rajanaidu *et al.*, 2000).

There are three varieties of oil palm, namely dura, pisifera and tenera, with the last one preferred in economic plantations throughout Southeast Asia (Muniran, Bhore, & Shar, 2008). Tenera variety is a hybrid between dura and pisifera, and is characterized by thick mesocarp layer, relatively thin shell (0.5-3 mm), and higher oil yield than dura or pisifera (Corley & Tinker, 2003; Jalani, Cheah, Rajanaidu, & Darus, 1997; Jin et al., 2016; Rajanaidu et al., 2000). In the production of tenera oil palm, dura and pisifera parents are crossed and the tenera progenies are subsequently tested for adaptability and stability, which are prime considerations in breeding programs of tree crops (Shukla et al., 2015). Therefore, multi-location trials are crucial in an oil palm breeding program, to evaluate agronomic stability and adaptability of new varieties in various environments. The dura and pisifera parents of tenera hybrids are selected for wide adaptation and yield stability, in the commercial

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production of tenera seedlings (Noh et al., 2014; Rafii et al., 2012). Stability analysis of tree crop is generally time consuming as maturation of the plant takes several years. Oil palm typically begins to produce fruit three years after planting, hence a conventional breeding scheme of oil palms can take 8-10 years per cycle, with the majority of this time spent in yield measurements of the tenera hybrids (Soh, Wong, Hor, Tan, & Chew, 2003). Additionally, including more plants in the analysis increases the expenses for labor and space needed. Therefore, preliminary information regarding the genetic variations in the populations to be studied is valuable, as it reflects the adaptive capacity of the plants and helps design the breeding plan properly. Furthermore, it is advantageous to manipulate and exploit in breeding the genetic relationships of the plant materials in a breeding program, and subsequently in productive field use. The measurement of genetic variations in seedling stage possibly facilitates breeding based on predicted adaptability and stability of the tenera hybrids from each individual cross. However, variations in the morphological traits can be influenced by environmental factors (Ganesan, Singh, Choudhury, Bharadwaj, & Singode, 2014). Using morphological along with molecular approaches provides better insights into the genetic diversity within and between populations. Among the molecular techniques currently available for the assessment of genetic diversity, SSR markers are the most common and frequently used owing to quickness, simplicity, richness in polymorphism, and stability. The SSR markers were successful in assessment of genetic diversity in commercial and natural oil palm populations worldwide (Solin & Sobir, 2014; Taeprayoon, et al., 2015; Zhou, Xiao, Xia, & Yang, 2015). In this present study, twelve improved tenera hybrid populations derived from crossing twelve different maternal dura and one paternal pisifera were assessed for genetic variability based on morphological and SSR markers.

### 2. Materials and Methods

## 2.1 Plant materials

Twelve F1 tenera hybrid populations from crossing twelve different selected dura (female parent) palms and one common *pisifera* (male parent) palm were developed at the Prince of Songkla University, Songkhla, Thailand, and are referred to as P1, P2, and P4 to P13. The genetic materials used for developing the studied populations were originally F2 (tenera×tenera) populations collected from plantations back in 1987 and have been through breeding and selection processes (Eksomtramage et al., 2001). The genetic backgrounds of the mentioned germplasms were Deli dura and AVROS pisifera imported from Malaysia during 1980s-1990s as tenera hybrid to southern Thailand by a commercial company (Eksom tramage, 2011). The F1 tenera hybrid seeds were processed for dormancy breaking. Briefly, pericarps were removed from the fruit and the naked seeds were then kept at 40 °C for 60 days in a temperature-controlled room. Subsequently, the sprouting oil palm seeds were sown in nursery trays under a shade net for 3 months, and the seedlings were then transferred to plastic bags and placed under full sunlight with daily water. Twelve oil palm seedlings supposed to represent limited number of alleles at the studied loci of controlled pollination populations were randomly selected for genetic variation analysis.

## 2.2 Morphological data

Twelve seedling samples per family according to completely randomized design were used to record the morphological data. Eight morphological characters, namely plant height, stem diameter, leaf width, leaf length, leaflet width, leaflet length, rachis length, and number of pinnate leaves, were recorded three months after transplanting to plastic bags.

## 2.3 Genomic DNA extraction

DNA was extracted using the CTAB method from approximately 300 mg of young leaves sampled from each of the tagged seedlings. Leaf tissue in liquid nitrogen was ground using mortar and pestle and transferred to a microcentrifuge tube. A total of 700  $\mu$ l of CTAB extraction buffer with 2%  $\beta$ mercaptoethanol was added to the tube and the mixture was then incubated at 60 °C for an hour. Subsequently, 800  $\mu$ l of chloroform was added to the tube prior to centrifugation at 13,000 rpm for 10 mins. The supernatant was transferred to a new microcentrifuge tube for DNA precipitation using 600  $\mu$ l of cool isopropyl alcohol. The DNA pellet was re-dissolved with 50  $\mu$ l of TE buffer (Doyle & Doyle, 1990). The purified genomic DNA was quantified on Nanodrop, and was subsequently diluted to 50 ng/ $\mu$ l for polymerase chain reaction.

#### 2.4 Amplification of microsatellite markers

The five microsatellite or SSR markers developed by Abdullah, Rafaii, Ithnin, Saleh, and Latif (2011) were used in the present study due to their ability to detect polymorphism among oil palms in Malaysian Palm Oil Board (MPOB) Research Station, the supposed source of genetic variability of improved oil palm population. Optimal annealing temperature for each primer was determined using gradient PCR. The SSR markers were amplified in a total of 12.5  $\mu l$  reaction mixture containing 1.25  $\mu \tilde{l}$  of 10x taq buffer, 0.25 µl of dNTP mixed (2mM each), 0.25 µl of 10 mM forward primer, 0.25 µl of 10 mM reverse primer, 1 µl of 25 mM MgCl2, 0. 0625 µl of taq polymerase, 8. 438 µl of Diethylpyrocarbonate (DEPC) water and 1 µl of 50 ng/µl DNA sample. The PCR amplification was performed as follows: pre-denaturation at 95 °C for 30 sec; 30 cycles of 30 sec denaturation at 95 °C, 30 sec annealing at 52-56 °C, and 30 sec extension at 68 °C; and final extension at 68 °C for 5 mins. (Table 1). The PCR products were analyzed using the Microchip Electrophoresis System (MCE-202 MultiNA; Shimadzu, Kyoto, Japan).

#### 2.5 Data analysis

Morphological data were subjected to analysis of variance (ANOVA) and the least significant differences were calculated at P < 0.01. Principal component analysis (PCA), scatter diagram, and correlation analysis were performed using R software. Polymorphism information content (PIC)

Table 1. Details of the SSR primers used

No.	Primers	TA (°C)	Sequence (F: forward, R: reverse)	Allele size (bp)	Repeat unit
1	CNH00887	52	F: TTATTGATTGATGCAAGATACAC R: TTGATAAAATACAAGAGATAGCA	165	(AT) <sub>9</sub>
2	CNH01617	52	F: TCTTTAATTTGTCGAGGATAATG R: ATGCAAGGTTTTGTTGAAACT	130	(CT) <sub>20</sub>
3	CNI01937	52	F: AACTGCAAATGAGACACAGAG R: TCCACCAGAGGAGGGGTTAGT	170	(AG) <sub>9</sub>
4	EAP 03160	52	F: AACGTGAGAGCCATAGAGATAG R: TAATAGAAACTAGACCCGACCA	175	(TATG) <sub>6</sub>
5	MF233056	52	F: CCGAATAGAAGAGGAAAGAATA R: AGGTTTGGTGGAGAAGTGTT	232	(CT) <sub>15</sub>

TA: temperature of amplification.

for each SSR was calculated using the following formula: PIC =  $1 - \Sigma Pi^2$ , where Pi is the frequency of the ith SSR, according to Smith *et al.* (2000). Jaccard's genetic distances of the individual *tenera* hybrids from each population were calculated based on SSR marker polymorphism.

#### 3. Results and Discussion

#### 3.1 Morphological analysis

The twelve hybrid populations showed significant differences in all the morphological traits studied (Table 2). P13 recorded the highest mean values in plant height, leaf width, leaf length and number of pinnate leaves. P6 showed superior leaflet length and rachis length. The lowest mean values in all traits were observed for the P5 population. High within-population variations as indicated by the standard deviation were observed for the P4 population in most traits. The P13 population showed highest variation of plant height and stem diameter traits, while the P1 population exhibited high variation in leaf width and leaflet width characters. To identify the most variant traits among the tenera hybrid populations, coefficients of variation were computed. Among the morphological traits studied, the highest coefficients of variation were for rachis length, leaflet width, and number of pinnate leaves. The diversity of these light-inception related characters might be attributed to variations in photosynthesis, which influences both biomass and yield of an oil palm (Corley, 1973; Gerritsma & Soebagyo, 1999).

Principal component analysis is an important multivariate technique used to assess the associations between traits and to measure the genetic diversity (Abdi & Williams, 2010). Principal component analysis of the eight morphological traits revealed that the first two components represent 76.5% of the total variation: the first and the second principal components (PC1 and PC2) accounted for 62. 48% and 14.02%, respectively (Table 3). All the variables contributed equally and in the same direction in PC1, whereas rachis length and leaflet length had strong contributions to PC2. Tan and Hardon (1976) reported that leaf traits measured at nursery stage were significantly correlated with many characters of mature oil palm, and suggested that selection based on leaf characters could therefore result in higher growth rate and yield of oil palm in the field. Besides, it has

been reported that the size of seedling transplanted to the field has a profound effect on the time when seedlings start to bear fruit. Therefore, the fast growing seedlings in nursery are preferred as they begin to produce fruit earlier (Corley, 1976; Hartley, 1977; Turner & Gillbank, 1974). Thus, hybrids with large PC1 and PC2 components could have high potential as parental dura and pisifera in the commercial production of high-performance tenera hybrids. However, in the development of so-called compact oil palm, small rachis length is preferred, so hybrids with high PC1 but low PC2 would be selected. Scatter diagram of PC1 and PC2 across the individual hybrids revealed that the hybrid populations were not well-separated and hybrids from each population were scattered in all the quadrants, indicating high levels of genotypic variation. However, the majority of hybrids from P1, P2, and P6 were located in quadrant 1, suggesting that these hybrids exhibited high performance traits (Figure 1).

Correlation analysis showed significant positive correlations of all pairs of traits, with the exception of rachis length and stem diameter. This conforms to the findings of Lucas (1980), who reported high positive correlations among seedling height, leaf number, and stem diameter. Subronto, Taniputra, and Manurung (1989) reported that bulb diameter and leaf area of oil palm seedlings are highly positively correlated (Table 4). Correlation studies can be used to find indirect or surrogate selection criteria to improve a trait of interest, with benefits when the surrogates are easier or faster to determine (Silva, Moro, Moro, Santos, & Buzinaro, 2016).

## 3.2 SSR-marker based analysis

Five SSR markers were amplified from the DNA of the 144 individual hybrids, and all these markers showed polymorphic bands (Figure 2). A total of 18 polymorphic bands from five markers were observed, with on average 3.6 alleles (Table 5). The number of alleles per locus ranged from 2 to 5, with the highest number count found with primers CNI01937 and MF233056. Abdullah *et al.* (2011) reported the highest number of alleles with primer MF233056 in a population of Deli *dura*, AVROS *pisifera*, and F<sub>1</sub> *tenera* hybrids, in Malaysia, indicating high level of polymorphism at this locus. PIC for each marker ranged from 0.4042 to 0.7965 with an average value of 0.6211, demonstrating high information content.

Table 2.	Mean and standard deviation	for eight morphological	traits within each of the	twelve <i>tenera</i> hybrid populations

Popu- lation	Plant he	Plant height Stem dia		Stem diameter Le		Leaf width Leaf length		ıgth	Leaflet width		Leaflet length		Rachis length		No. of pinnate leaves	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
P1	3.67 <sup>abcd</sup>	1.38	13.92 <sup>a</sup>	0.52	15.58 <sup>a</sup>	2.57	40.66 <sup>abcd</sup>	5.53	1.70 <sup>abc</sup>	0.41	22.83 <sup>ab</sup>	1.75	2.02 <sup>abc</sup>	0.48	6.0 <sup>bc</sup>	0.74
P2	3.35 <sup>cde</sup>	0.52	13.42 <sup>abc</sup>	0.36	14.00 <sup>abc</sup>	1.20	41.58 <sup>abcd</sup>	5.25	1.68 <sup>abc</sup>	0.24	22.00 <sup>abc</sup>	2.26	2.13 <sup>ab</sup>	0.48	4.9 <sup>de</sup>	0.67
P4	3.81 <sup>abc</sup>	1.93	12.92 <sup>abc</sup>	0.59	15.33 <sup>a</sup>	2.01	43.08 <sup>abc</sup>	6.95	1.97 <sup>a</sup>	0.41	22.67 <sup>ab</sup>	2.35	1.79 <sup>bc</sup>	0.36	5.5 <sup>bcd</sup>	1.08
P5	2.98 <sup>e</sup>	0.95	11.00 <sup>d</sup>	0.32	11.08 <sup>d</sup>	2.27	35.50 <sup>d</sup>	4.72	1.34 <sup>de</sup>	0.22	19.66 <sup>c</sup>	3.17	1.59 <sup>c</sup>	0.28	4.1 <sup>e</sup>	0.39
P6	3.43 <sup>bcde</sup>	1.11	12.17 <sup>bcd</sup>	0.24	13.17 <sup>bc</sup>	1.11	43.08 <sup>abc</sup>	5.60	1.61 <sup>bcd</sup>	0.30	23.42 <sup>a</sup>	1.62	2.35 <sup>a</sup>	0.57	5.0 <sup>cde</sup>	0.67
P7	3.23 <sup>de</sup>	1.11	11.83 <sup>cd</sup>	0.33	12.67 <sup>cd</sup>	1.62	37.33 <sup>bcd</sup>	4.48	1.38 <sup>cde</sup>	0.14	19.67 <sup>c</sup>	2.23	1.83 <sup>bc</sup>	0.39	4.9 <sup>de</sup>	0.90
P8	3.53 <sup>bcd</sup>	1.08	12.58 <sup>abc</sup>	0.33	12.92 <sup>bcd</sup>	1.24	38.91 <sup>abcd</sup>	3.58	1.42 <sup>cde</sup>	0.20	21.25 <sup>abc</sup>	2.67	1.88 <sup>bc</sup>	0.42	5.1 <sup>cd</sup>	0.72
P9	3.63 <sup>abcd</sup>	1.17	12.50 <sup>abcd</sup>	0.41	14.42 <sup>abc</sup>	1.44	43.33 <sup>ab</sup>	6.51	1.68 <sup>abc</sup>	0.35	$22.17^{abc}$	2.79	2.01 <sup>abc</sup>	0.35	5.9 <sup>bc</sup>	0.90
P10	3.35 <sup>cde</sup>	1.04	12.00 <sup>bcd</sup>	0.32	13.25 <sup>bc</sup>	1.29	36.67 <sup>cd</sup>	2.84	1.28 <sup>e</sup>	0.20	20.33 <sup>bc</sup>	2.50	1.68 <sup>bc</sup>	0.30	5.4 <sup>bcd</sup>	0.90
P11	3.63 <sup>abcd</sup>	1.38	12.58 <sup>abc</sup>	0.35	14.00 <sup>abc</sup>	1.28	42.17 <sup>abc</sup>	5.80	1.54 <sup>cde</sup>	0.17	21.42 <sup>abc</sup>	2.68	1.72 <sup>bc</sup>	0.30	5.25 <sup>bcd</sup>	0.96
P12	3.89 <sup>ab</sup>	1.17	12.92 <sup>abc</sup>	0.28	14.75 <sup>ab</sup>	1.14	43.41 <sup>ab</sup>	5.44	1.68 <sup>abc</sup>	0.20	$20.92^{abc}$	1.57	1.74 <sup>bc</sup>	0.28	6.1 <sup>b</sup>	0.39
P13	4.09 <sup>a</sup>	2.02	13.50 <sup>ab</sup>	0.65	15.33 <sup>a</sup>	1.92	44.83 <sup>a</sup>	6.21	1.92 <sup>ab</sup>	0.24	21.16 <sup>abc</sup>	1.99	1.73 <sup>bc</sup>	0.22	7.0 <sup>a</sup>	1.08
F-test	**		**		**		**		**		**		**		**	
C.V.	11.56		10.30		11.97		13.12		16.98		10.93		20.52		14.89	

\*\* Significant difference at  $P\!\leq\!0.01$ 

Table 3. Eigenvector coefficients of 8 morphological traits in their 8 principal components, along with eigenvalue, and individual and cumulative percentages of the total variance

Characters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Plant height	0.377	-0.077	-0.389	-0.367	-0.583	-0.049	-0.275	-0.383
Stem diameter	0.364	-0.388	-0.234	0.010	0.051	0.051	-0.260	0.768
Leaf width	0.390	-0.143	0.269	-0.044	-0.263	-0.387	0.722	0.113
Leaf length	0.393	0.098	0.001	-0.261	0.254	0.773	0.312	-0.095
Leaflet width	0.366	0.009	0.510	0.607	-0.277	0.220	-0.313	-0.124
Leaflet length	0.330	0.432	0.411	-0.454	0.310	-0.328	-0.348	0.077
Rachis length	0.212	0.729	-0.474	0.375	-0.049	-0.090	0.136	0.174
No. of pinnate leaves	0.363	-0.310	-0.261	0.283	0.590	-0.291	-0.015	-0.437
Eigenvalue	4.999	1.122	0.502	0.381	0.310	0.249	0.225	0.211
Variance percent	62.488	14.024	6.278	4.760	3.877	3.113	2.818	2.641
Cumulative variance percent	62.488	76.512	82.790	87.550	91.427	94.541	97.359	100.000

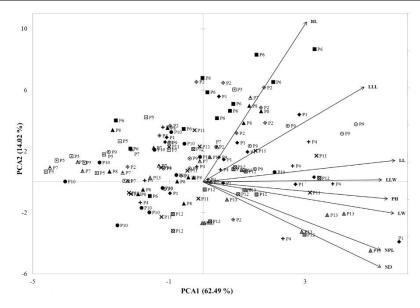


Figure 1. Scatter diagram of the first two principal components based on genotype and eight morphological attributes, for 144 progenies total from twelve *tenera* hybrid populations

 Table 4.
 Correlation matrix of morphological traits in the *tenera* hybrid seedlings

	Plant height	Stem diameter	Leaf width	Leaf length	Leaflet width	Leaflet length	Rachis length	No. of pinnate leaves
Plant height	1.000							
Stem diameter	0.708**	1.000						
Leaf width	0.700**	0.707**	1.000					
Leaf length	0.701**	0.651**	0.708**	1.000				
Leaflet width	0.581**	0.600**	0.718**	0.659**	1.000			
Leaflet length	0.531**	0.395**	0.588**	0.675**	0.585**	1.000		
Rachis length	0.365**	0.144ns	0.265**	0.444**	0.346**	0.534**	1.000	
No. of pinnate leaves	0.656***	0.762**	0.684**	0.648**	0.604**	0.419**	0.215**	1.000

\*\*, \*\*\*Significant by the T test at P $\leq$  0.01 and P $\leq$  0.001, respectively

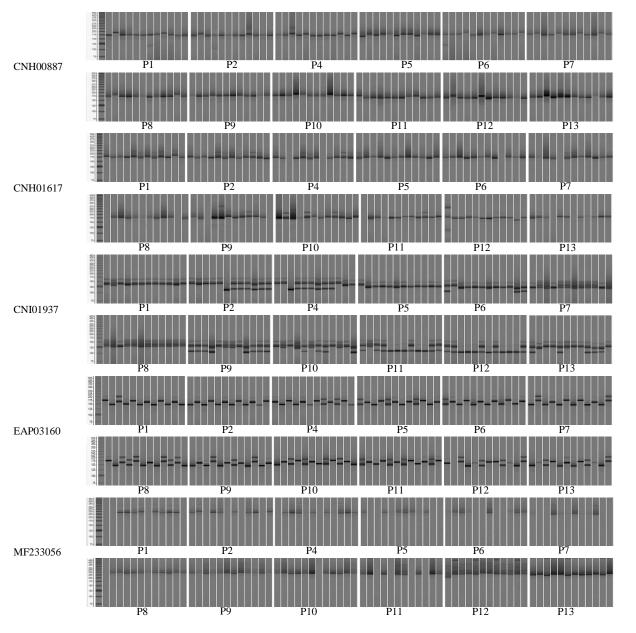


Figure 2. Amplification patterns obtained for five SSR markers (CNH00887, CNH01617, CNI01937, EAP03160, and MF233056) across 144 *tenera* hybrids in twelve different hybrid population

С
)49
)42
392
107
965
)58
211

Table 5. Number of alleles amplified and PIC for each SSR primer

The genetic distances of individual *tenera* hybrids in each population were calculated based on SSR marker polymorphisms (Table 6). From the mean and variance of genetic distances, P1, P4, P6, and P8 showed the highest variations, as they did in the morphological traits. This indicates that *dura* and *pisifera* parents of P4 were the most diverse, as according to Corley, Hughes, Jack, Batty, & Mayes (1992) those oil palm parental lines with greater genetic distance generate more variable progenies. The smallest genetic distance for *tenera* hybrids was 0.00, observed in P1, P4, P5, P6, and P8.

Table 6. Summary statistics by population of genetic distances based on SSR polymorphisms

D1-+'		Genetic	distance	
Population	Max	Min	Mean	Variance
P1	0.3317	0.0000	0.2169	0.0034
P2	0.3317	0.1000	0.2412	0.0022
P4	0.3606	0.0000	0.2599	0.0040
P5	0.2828	0.0000	0.1980	0.0026
P6	0.3162	0.0000	0.2232	0.0029
P7	0.3162	0.1000	0.2223	0.0024
P8	0.2828	0.0000	0.1956	0.0029
P9	0.3162	0.1000	0.2871	0.0021
P10	0.3162	0.1414	0.2236	0.0021
P11	0.3316	0.1414	0.2516	0.0018
P12	0.2828	0.1000	0.2078	0.0021
P13	0.3000	0.1000	0.2112	0.0019

## 4. Conclusions

Both morphological and SSR analysis indicated genetic variations in all the *tenera* hybrid populations of the present study. However, P1, P4, P6, P8, and P13 showed the most within population genetic variations among the studied populations. Therefore, based on these results, P1, P4, P6, P8, and P13 *tenera* hybrid populations offer high potential to provide wide adaptation and yield stability, and should be subjected to further stability analysis, in support of oil palm breeding programs.

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